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One significant effect did occur indicating an increase in glutathione reductase post exposure for both the control group and acid group.

This report covers a period from October 23, 1978, to December 15, 1978, and work was completed as of May 1, 1979.

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BIOCHEMICAL CHANGES IN HUMANS UPON EXPOSURE TO SULFURIC ACID AEROSOL AND EXERCISE

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This report has been reviewed by the Health Effects Research Laboratory, U.S. Environmental Protection Agency, and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

FOREWORD

The many benefits of our modern, developing, industrial society are accompanied by certain hazards. Careful assessment of the relative risk of existing and new man-made environmental hazards is necessary for the establishment of sound regulatory policy. These regulations serve to enhance the quality of our environment in order to promote the public health and welfare and the productive capacity of our Nation's population.

The Health Effects Research Laboratory, Research Triangle Park, conducts a coordinated environmental health research program in toxicology, epidemiology, and clinical studies using human volunteer subjects. These studies address problems in air pollution, non-ionizing radiation, environmental carcinogenesis and the toxicology of pesticides as well as other chemical pollutants. The Laboratory participates in the development and revision of air quality criteria documents on pollutants for which national ambient air quality standards exist or are proposed, provides the data for registration of new pesticides or proposed suspension of those already in use, conducts research on hazardous and toxic materials, and is primarily responsible for providing the health basis for non-ionizing radiation standards. Direct support to the regulatory function of the Agency is provided in the form of expert testimony and preparation of affidavits as well as expert advice to the Administrator to assure the adequacy of health care and surveillance of persons having suffered imminent and substantial endangerment of their health.

Since sulfuric acid is a part of our modern environment, it becomes necessary to establish if any responses are observed in humans under controlled exposure conditions. This study was designed to further define the possible, irritant effects of sulfuric acid mist on human health.

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ABSTRACT

A total of 18 human subjects were exposed to ambient air for four hours on the first day of exposure and to four hours of 100 ug/m^3 (0.033 uM) sulfuric acid aerosol (0.5 um mean mass diameter) on the second day. A total of 17 human subjects were exposed to four hours of ambient air on both exposure days.

Six biochemical blood parameters were measured pre and post exposure: glutathione, lysozyme, glutathione reductase, serum gultamic oxaloacetic acid transaminase, serum vitamin E and 2,3-diophosphoglyceric acid. The results indicate no significant effect of one four hour exposure of humans to sulfuric acid aerosol (100 ug/m^3).

One significant effect did occur indicating an increase in glutathione reductase post exposure for both the control group and acid group.

This report covers a period from October 23, 1978, to December 15, 1978, and work was completed as of May 1, 1979.

INTRODUCTION

Studies have shown that sulfuric acid and particulate sulfates are formed by the oxidation of a portion of the sulfur dioxide emitted into the atmosphere.[1] Experimental toxicology studies have suggested that these oxidation products have a greater irritant potency than sulfur dioxide gas per se.[2] Human sulfuric acid exposure studies have concentrated mainly on the retention of inhaled acid mist as a function of particle size and concentration and the magnitude of the response as measured by pulmonary function effects.[3,4,5,6,7] Few studies have been reported that evaluate effects of sulfuric acid mist on biochemical blood parameters. Alarie et al.[8] exposed cynomolgus monkeys and guinea pigs to sulfuric acid mist for 78 weeks and 52 weeks, respectively. No deleterious effects due to sulfuric acid mist on the measured biochemical blood parameters could be detected. Since sulfuric acid is a part of our modern environment, it becomes necessary to establish if any responses are observed in humans under controlled exposure conditions. This study was designed to further define the possible irritant effects of sulfuric acid mist on human health. Six biochemical blood parameters were evaluated in humans exposed to sulfuric acid mist.

METHOD

Subjects

A total of 35 healthy (as determined by a physical examination and completion of the Duke University Computer History form and the Minnesota Multiphasic Personality Inventory), non-smoking, Caucasian, male, university students characterized by an average height of 179.8 cm, a standard

deviation of 7.2 cm, a range of 160.0-195.0 cm, an average weight of 72.6 kg, a standard deviation of 8.6 kg, a range of 56.6-95.5 kg, and an average age of 28.0 years, a standard deviation of 3.5 years, a range of 21.7-34.4 years, were used in this study. A total of 17 subjects were exposed to air only (group 1, controls) and a total of 18 subjects were exposed to sulfuric acid aerosol (group 2, experimental).

Procedure

Each subject served for 2 days. On each day blood was drawn immediately preceeding a 4 hour exposure and immediately following the exposure. During the exposure, a pulmonary function battery was administered at 0, 2, and 4 hours. During the first 15 minutes, minute ventilation was recorded. At 30 minutes and 90 minutes, the subjects engaged in a 15 minute exercise period. This consisted of walking 4 mph on a treadmill inclined at 10°. Subjects were tested in groups of three.

Experimental Design

All subjects were exposed to ambient air on the first day of exposure and 18 of the subjects were exposed to 100 $\mu g/m^3$ (0.033 μM) H_2SO_4 aerosol (0.5 μm mean mass diameter, MMD) on the second day. The remaining 17 subjects received ambient air on the second exposure day. Data were collected in a counterbalanced fashion. During week one, a group of 3 acid exposure subjects was tested on Monday and Tuesday and a group of 3 air exposure subjects was tested on Wednesday and Thursday. The following week the order was reversed. Exposures began at approximately 8:30 a.m. each morning. This alternation scheme was continued throughout the experiment. This scheme helped control any day of week effects and time of year effects.

The chamber atmosphere was maintained at a temperature of 22°C, a relative humidity of 40% and an air flow of 227 m^3/min.

A total of six serum and red blood cell biochemical measures were chosen as dependent variables: serum glutathione (GSH), lysozyme, 2,3-diphosphoglycerate (2,3-DPG), serum glutamic oxaloacetic acid transaminase (SGOT), serum vitamin E, and red blood cell glutathione reductase. Measurements were taken on all six variables preceeding and following both exposure days. Each measurement was treated as a dependent variable in the analysis. Use of multivariate analysis of variance (MANOVA) allowed evaluating differences between (1) the air and acid groups, (2) day 1 and day 2 measurements, (3) pre and post measurements, and (4) all interactions of these effects.

Blood Analysis

SGOT was analyzed on the Centrifichem 400 autoanalyzer by a modified Karmen technique.[9,10] Red blood cell glutathione reductase was assayed by the method of Nichoalds[11] modified such that the enzyme was preincubated at 0°C for 30 minutes in the presence of 60 μM FAD and the reaction was initiated by the simultaneous addition of 2 mM oxidized glutathione (GSSG) and 0.24 mM NADPH. 2,3-DPG in erythrocytes was measured by the Mygaard and Rorth[12] method. Reduced glutathione in serum was measured by the method of Patterson and Lazarow.[13,14] Serum vitamin E was measured by the method of Chaney et al.[15] Lysozyme was measured by the method of Shugar.[16]

Materials

A Centrifichem Model 400 centrifugal analyzer and Centrifichem autopipetter (Union Carbide Corporation, Clinical Diagnostics, Rye, New York 10580) were used for assaying SGOT. Manual assays for glutathione reductase, GSH, and 2,3-DPG were run on a Cary 118 spectrophotometer. The lysozyme assay was run on a Varian 635 spectrophotometer. SGOT reagent kits were purchased from Union Carbide Corporation, Clinical Diagnostics, Rye, New York 10580. 2,3-DPG reagent kits were purchased from Calbiochem, La Jolla, California 92037. Lysozyme reagent kits were purchased from Worthington Diagnostics, Freehold, New Jersey 07728.

D,L-α-tocopherol, GSSG (free acid, grade III), FAD (disodium salt, grade III), NADPH (tetrasodium salt, Type 1), alloxan monohydrate, and GSH (reduced form, 98-100% purity) were purchased from Sigma Chemical Company, St. Louis, Missouri 63178.

RESULTS

Table 1 provides summary statistics for all six dependent variables. Data from both the air-air and air-acid treatment groups are included. None of the variables appear to pose any problems in terms of violating assumptions necessary for the analysis of variance.

Table 2 summarizes the MANOVA computed for these data. Each line in this table tests for an effect on any of the six dependent variables, or any combination of the six simultaneously. The important hypotheses tested involve the air-acid difference. None of those effects were significant. The overall pre-post effect, however, was significant ($p < 0.05$). Of the six univariate tests only glutathione reductase had

a significant effect as indicated by a p value of 0.001. No other variable had a pre-post p value less than 0.14. The mean for glutathione reductase pre-exposure was 7.27 μmoles/gm Hgb/min and the post-exposure mean was 7.76 μmoles/gm Hgb/min. This pre to post increase produced the significant p value.

Table 3 provides mean response for all six dependent variables at each point in time for each exposure group. These means were estimated as part of the MANOVA. The same data are presented graphically in Figures 1 through 6. All variables except RBC glutathione indicate nonsignificant changes. These figures support this conclusion, indicating that the exposed group and control group started close together and remained close together during the experiment. The significant pre-post effect for glutathione reductase is easily seen in Figure 3. When viewing the graph it should be remembered that day 1 was an air exposure for both groups.

DISCUSSION

The results indicate no effect on the blood parameters measured of one 4 hour exposure of humans to 100 $\mu g/m^3$ (0.033 μM) H_2SO_4 aerosol (0.5 MMD). This study considered biochemical blood parameters that are involved in maintaining cellular reductive detoxification ability which in turn protects cellular components from oxidation and the blood lysozyme level which is an indicator of lung tissue damage.

One significant effect did occur indicating an increase in the GSH reductase post-exposure. This effect was seen in both the air and acid exposure groups for each day of the exposure. Consequently acid exposure

is eliminated as a possible cause of the increase. A plausible hypothesis, not testable with this data, is that subjects were responding to the moderate level of exercise required during the exposure periods.

These results are consistent with the available animal studies. What few effects that have been seen have been at much higher levels of sulfuric acid (0.38 to 4.79 mg/m^3). Furthermore these effects have been pulmonary function effects. In fact, the effects may be confined to the respiratory system. Petering and Shih[17] have suggested that the conversion of sulfites to sulfates probably is a protective mechanism which occurs in the respiratory tract.

The collection schedule for this study was determined by the associated pulmonary function testing schedule. The scheme used would not allow detection of an effect unless it were detectable immediately post-exposure. With this one disadvantage, this research does not support the existence of any effect of exposure to sulfuric acid aerosol on any of the biochemical blood parameters measured.

Theoretically, sulfuric acid might be expected to initiate a response in the respiratory system due to its low pH (less than pH 1). However, the ammonia released by the respiratory system may well eliminate any effect of inhaled sulfuric acid aerosol through partial or complete neutralization.[18] Ammonia concentrations ranging from 29 $\mu g/m^3$ to approximately 2,200 $\mu g/m^3$ have been measured in exhaled air of healthy human adults. Stoichiometrically, ammonia at 1 $\mu g/m^3$ can convert sulfuric acid at a concentration of 5.8 $\mu g/m^3$ to ammonium bisulfate and at a concentration of 2.9 $\mu g/m^3$ to ammonium sulfate.[19] Thus 35 μg ammonia/m^3

would convert 100 μg/m^3 sulfuric acid to ammonium sulfate and only 17 μg ammonia/m^3 would convert this concentration of sulfuric acid to ammonium bisulfate. Consequently the amount of acid aerosol presented was probably neutralized in the respiratory tract. In recent studies on several animal species, on healthy humans and on human asthmatics, there has been no convincing evidence of functional changes upon exposure to ammonium bisulfate and ammonium sulfate. No effects were observed even at concentrations up to several milligrams per cubic meter.[20] Thus it appears that conversion of sulfuric acid to ammonium bisulfate or to ammonium sulfate does constitute an effective defense mechanism.

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20. Ibid, p. 7-61.

Table 1
Summary Statistics for All Six Dependent Variables

Time	Mean	Std. Dev.	Min.	Max.	Skewness
		Glutathione mg/100 ml			
Pre Day 1	41.53	4.91	33.60	54.80	0.74
Post Day 1	40.83	5.27	30.00	52.30	-0.03
		Lysozyme µg/ml			
Pre Day 1	10.97	2.89	5.49	17.94	0.34
Post Day 1	10.84	2.71	6.01	17.22	0.33
		Glutathione Reductase µmole/gm Hgb/min			
Pre Day 1	7.08	1.19	4.95	10.21	0.51
Post Day 1	7.70	1.20	5.98	11.52	0.98
		SGOT IU/l			
Pre Day 1	13.2	3.44	7.0	21.0	0.70
Post Day 1	12.9	2.97	9.0	21.0	1.05
		Serum Vitamin E µg/ml			
Pre Day 1	7.40	2.23	3.28	15.90	0.20
Post Day 1	7.43	2.71	3.05	18.14	1.76
		2,3 DPG µmole/ml RBC			
Pre Day 1	4.60	0.63	3.23	6.16	0.04
Post Day 1	4.79	0.79	3.86	7.72	2.07

Table 2
MANOVA Summary for All Six Dependent Variables

Source	Likelihood Ratio	~ F	Num df	Den df	p
Air/Acid (A)	.935	.32	6	28	.919
Day (D)	.766	1.43	6	28	.239
Pre-post (P)	.652	2.49	6	28	.046*
A x D	.770	1.39	6	28	.252
A x P	.926	.37	6	28	.890
D x P	.886	.60	6	28	.727
A x D x P	.737	1.67	6	28	.166

*Significant at the .05 level.

Table 3
Mean Response on All Six Dependent Variables
Estimated from MANOVA

Variable	Treatment	Day 1 Pre	Day 1 Post	Day 2 Pre	Day 2 Post
Glutathione mg/100 ml	Air-Air	40.93	41.85	42.08	38.99
	Air-Acid	42.10	39.87	41.36	42.54
Lysozyme µg/ml	Air-Air	11.33	11.21	11.44	11.42
	Air-Acid	10.63	10.48	11.02	11.12
Glutathione Reductase µmole/gm Hgb/min	Air-Air	6.86	7.63	7.38	7.84
	Air-Acid	7.29	7.77	7.56	7.81
SGOT IU/l	Air-Air	13.4	13.0	13.3	13.5
	Air-Acid	13.1	12.7	12.9	12.7
Serum Vitamin E µg/ml	Air-Air	7.36	7.39	7.28	7.74
	Air-Acid	7.43	7.47	6.86	6.68
2,3 DPG µmole/ml RBC	Air-Air	4.53	4.95	4.93	5.08
	Air-Acid	4.66	4.63	4.66	4.89

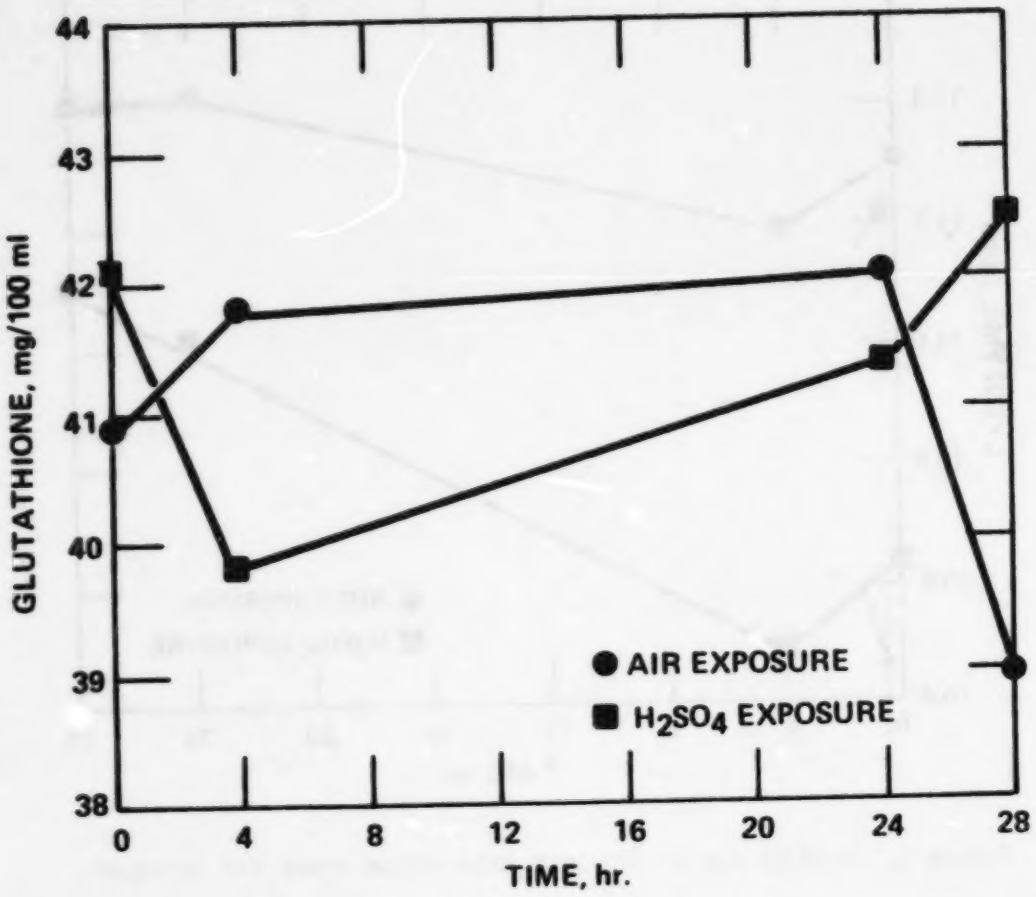

Figure 1. Acid by day by pre-post interaction means for GSH.

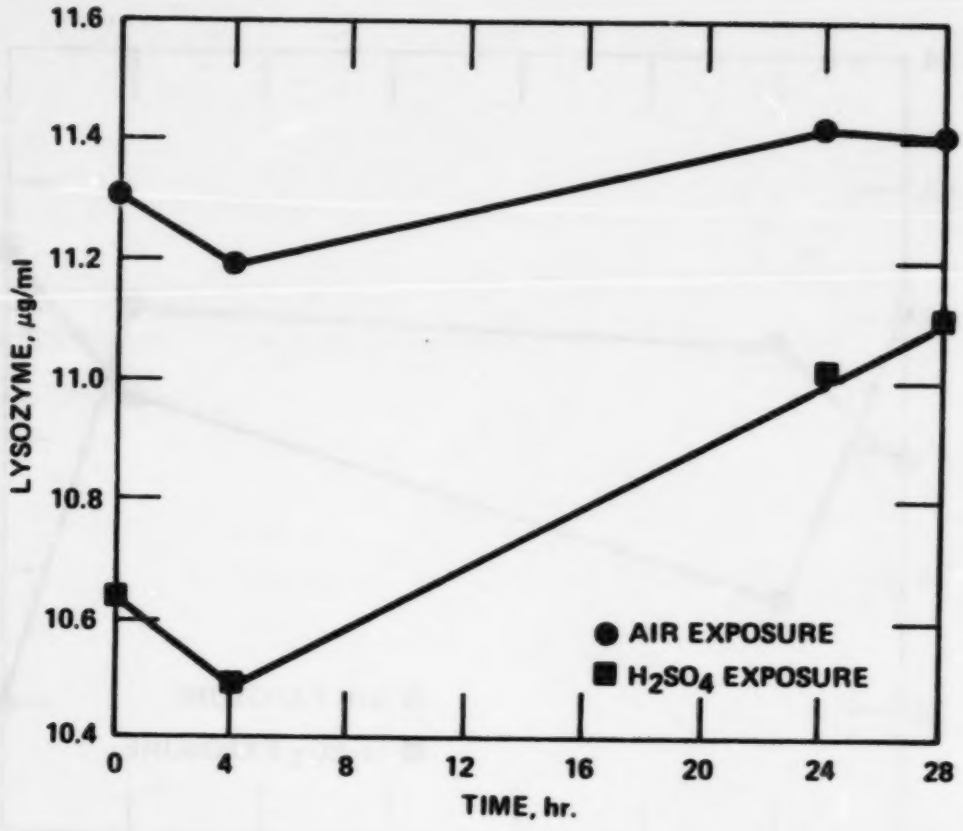

Figure 2. Acid by day by pre-post interaction means for Lysozyme.

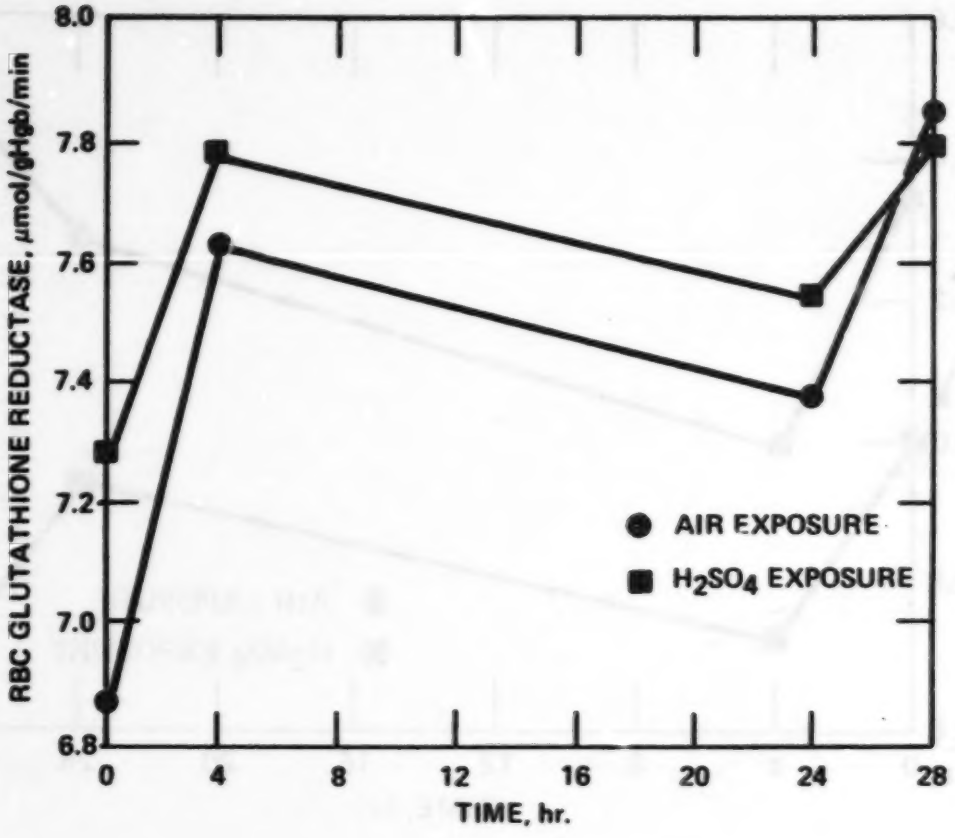

Figure 3. Acid by day by pre-post interaction means for RBC GSH Reductase.

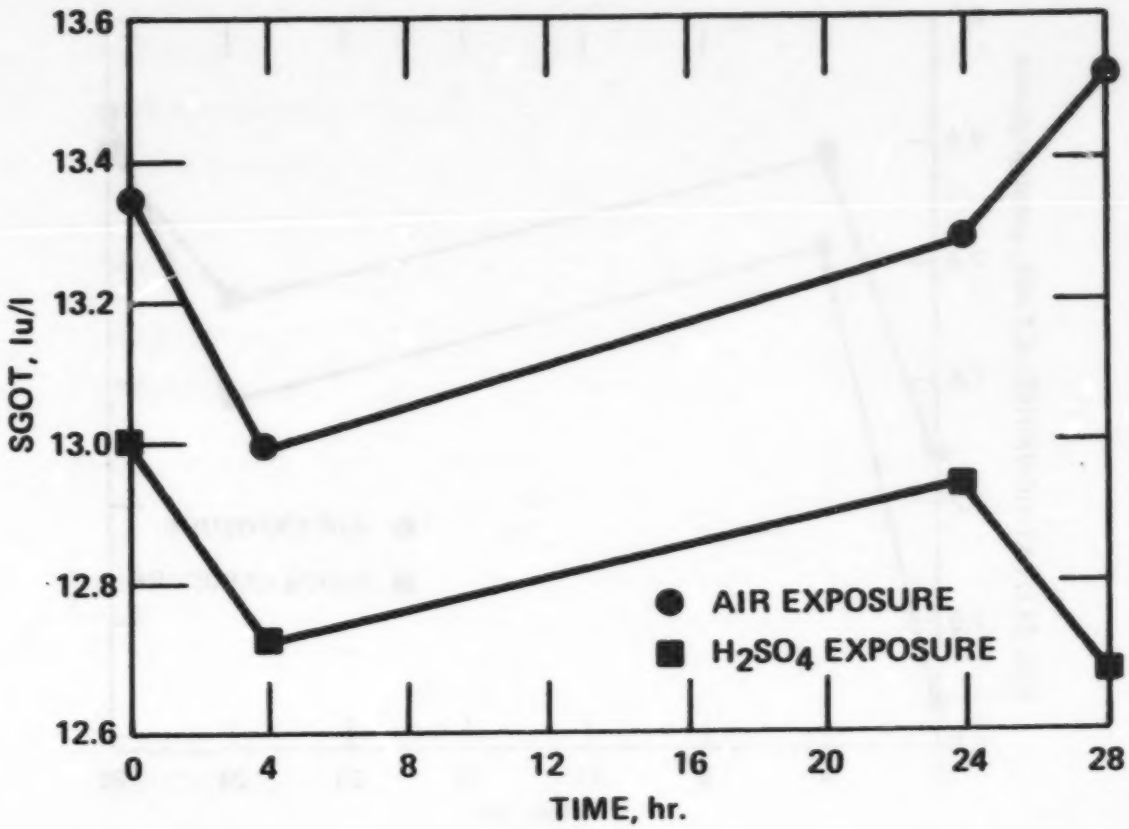

Figure 4. Acid by day by pre-post interaction means for SGOT.

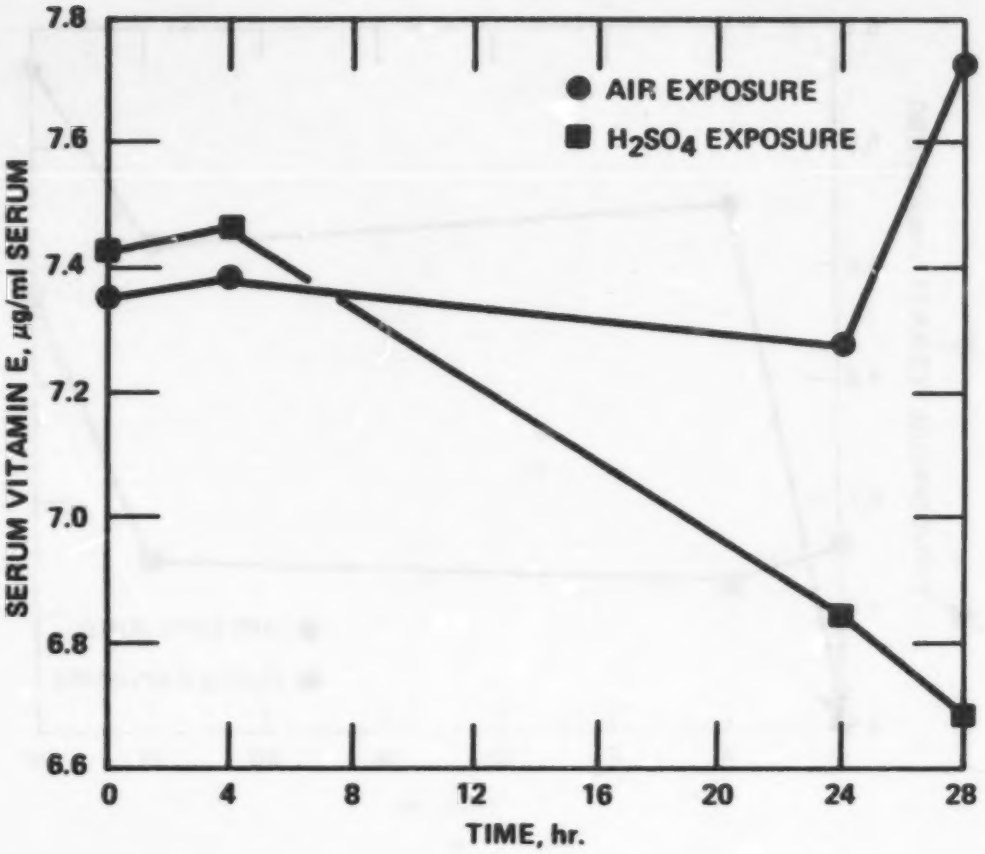

Figure 5. Acid by day by pre-post interaction means for Serum Vitamin E.

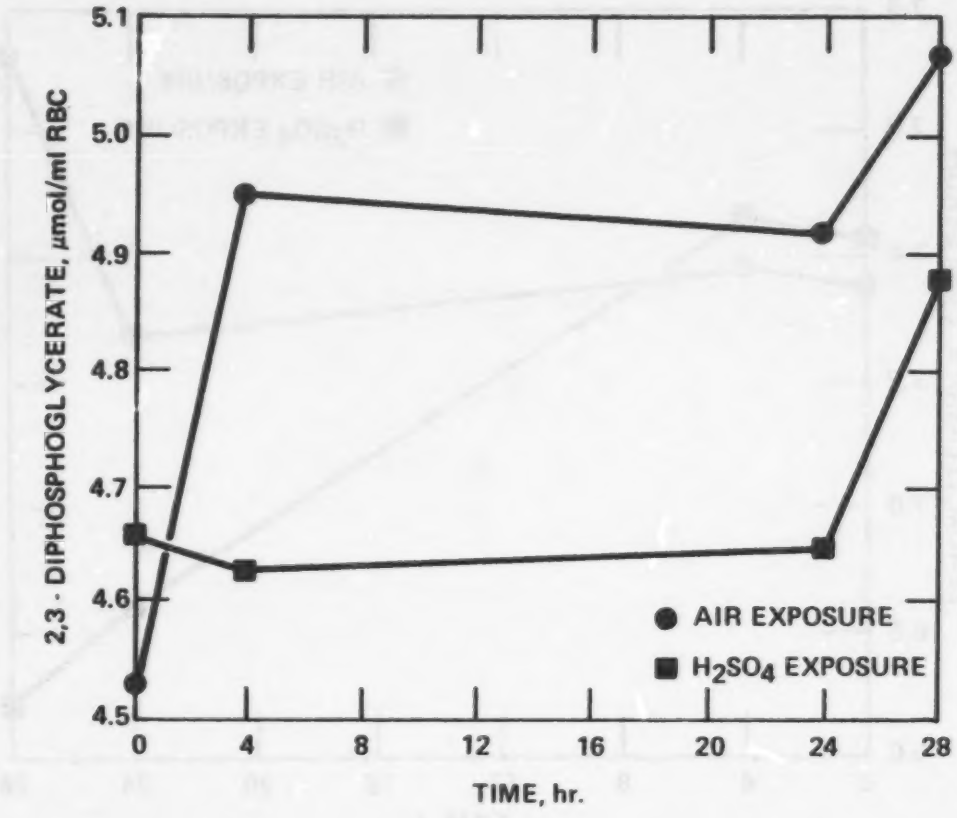

Figure 6. Acid by day by pre-post interaction means for 2,3-DPG.